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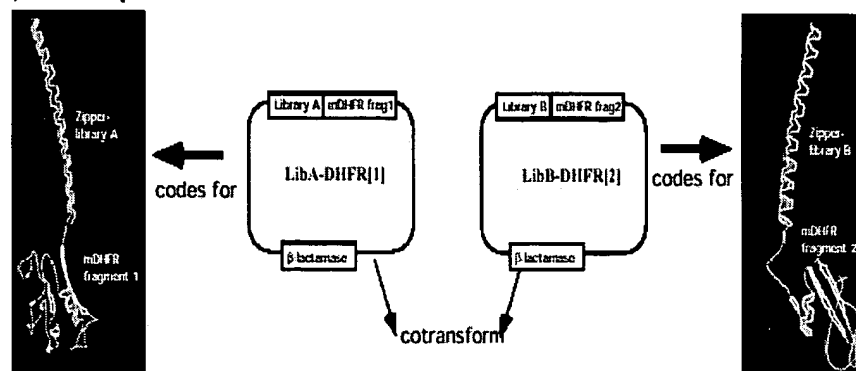
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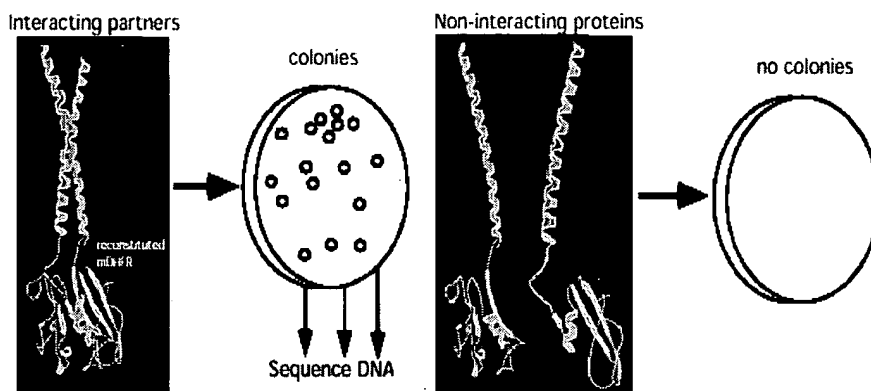
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Figure 1

(A) Library constructs



(B) Single-step selection



(C) Competition selection

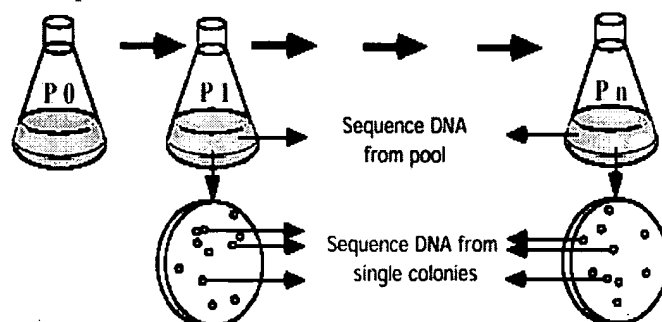


Figure 2

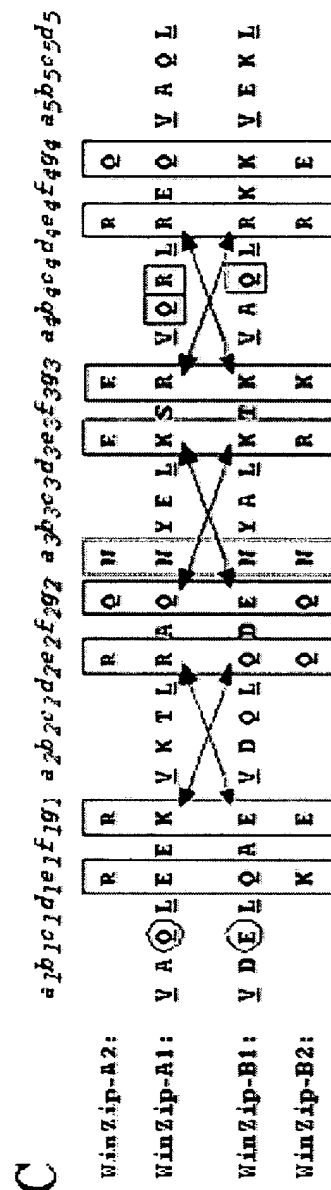
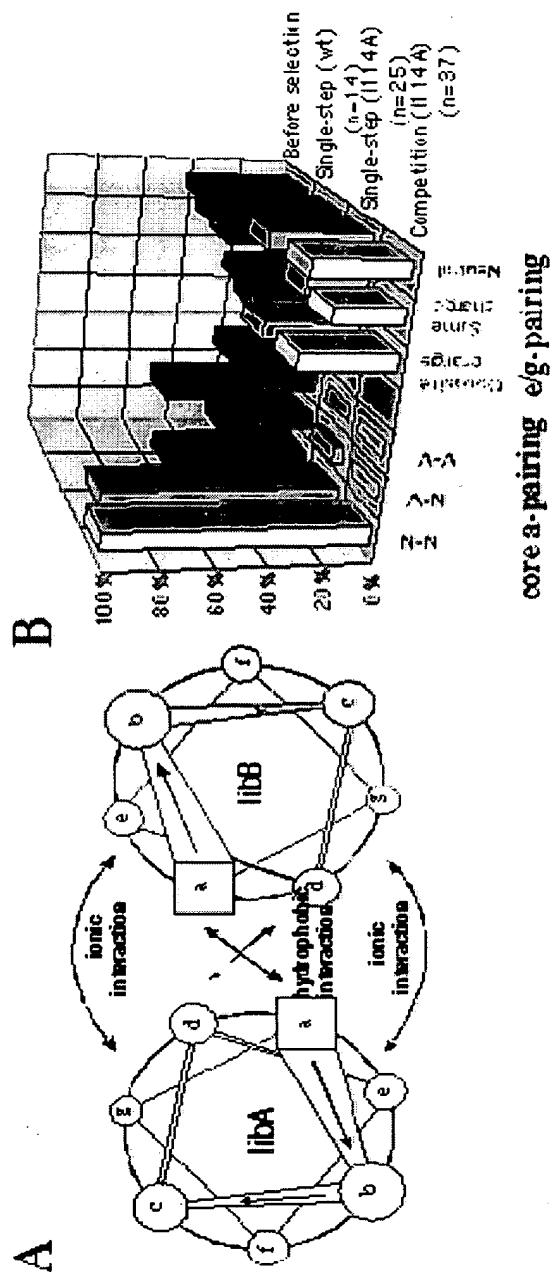


Figure 3

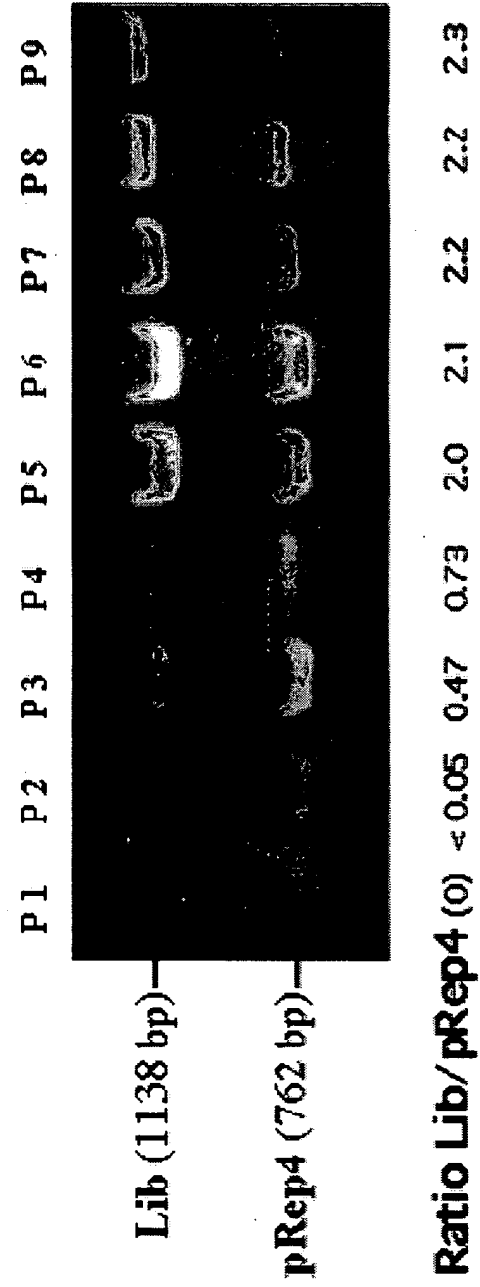


Figure 4

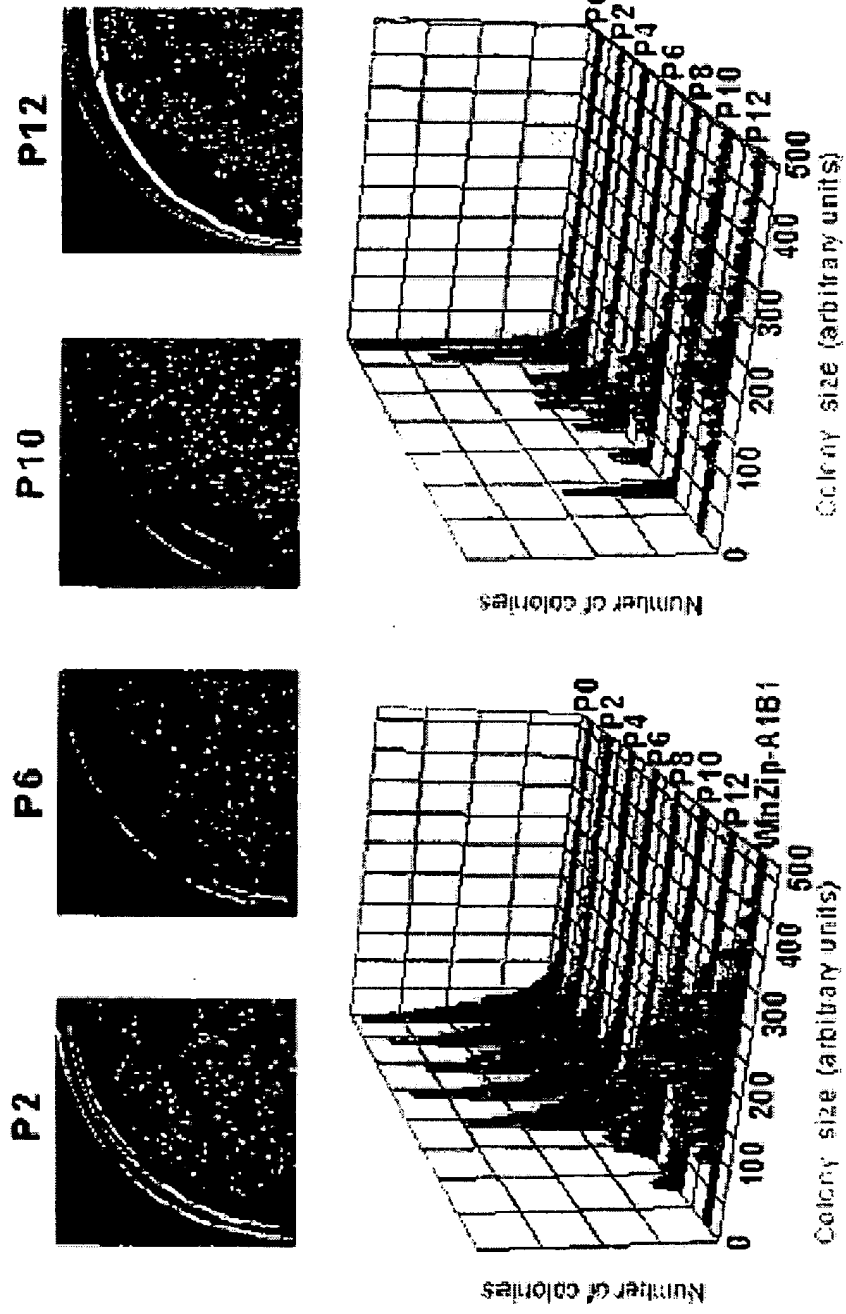


Figure 5

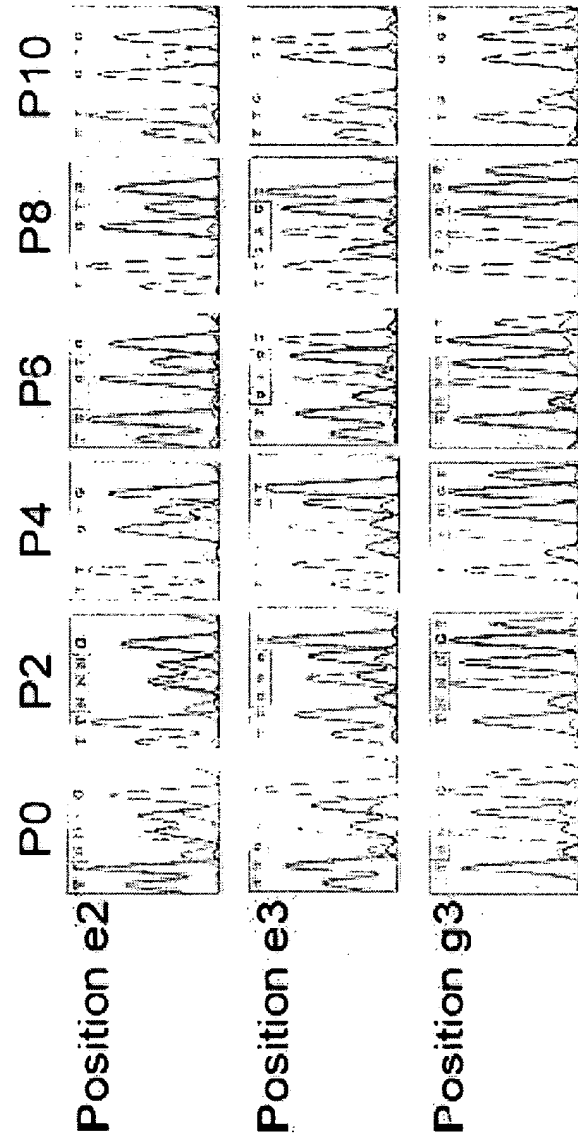
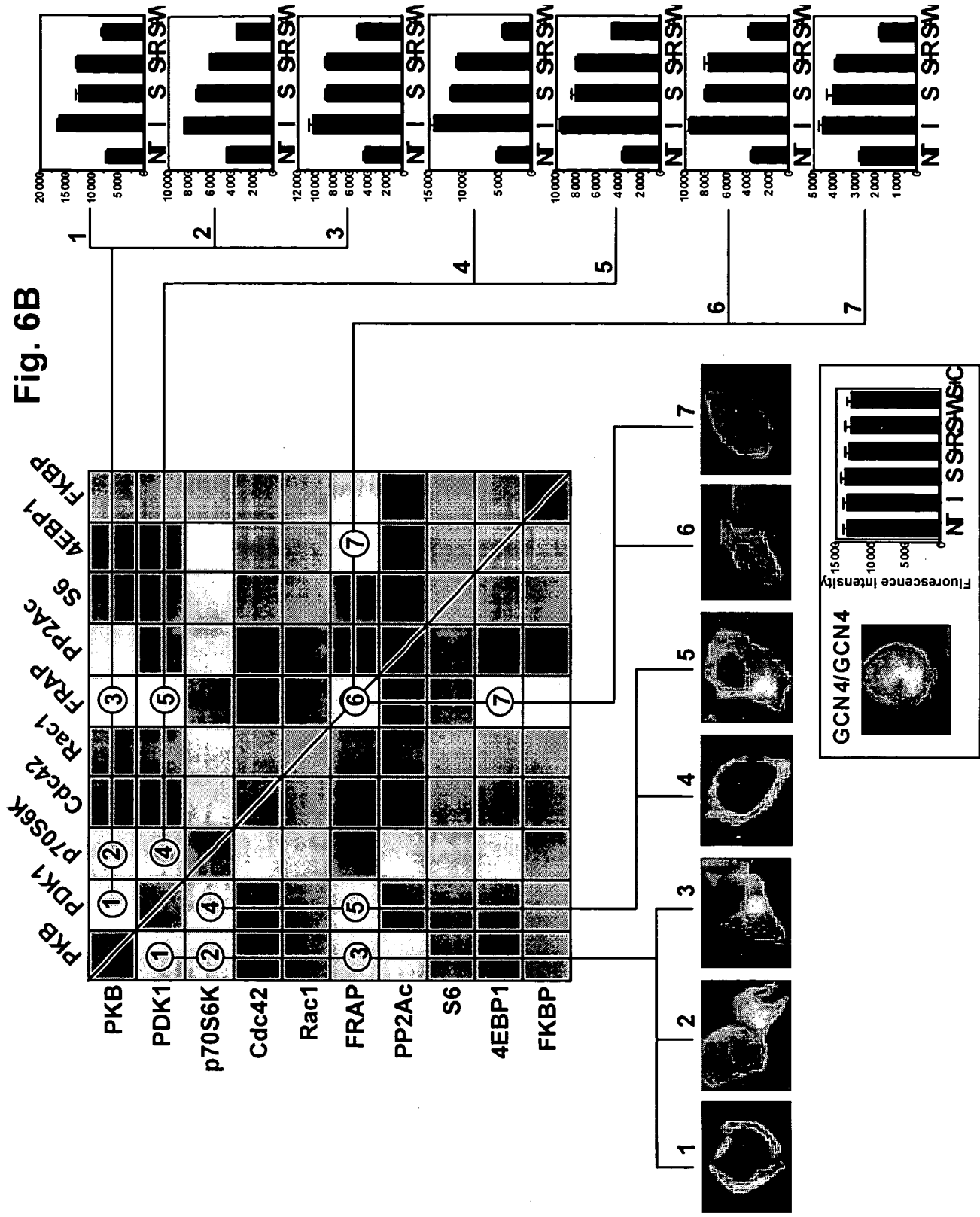


Fig. 6A

		Protein A		10aa		F[1,2]		or		10aa		F[1,2]		Protein A	
	PKB (WT)	PKB (K-A)	PDK1 (WT)	p70S6K (WT)	p70S6K (K-A)	Cdc42 (WT)	Rac1 (WT)	FRAP (WT)	FRAP (D-A)	PP2Ac (WT)	S6 protein (WT)	4EBP1 (WT)	FKBP (WT)		
	F[1,2]-X	F[1,2]-X	X-F[1,2]	X-F[1,2]	X-F[1,2]	F[1,2]-X	F[1,2]-X	F[1,2]-X	F[1,2]-X	X-F[1,2]	X-F[1,2]	X-F[1,2]	X-F[1,2]		
PKB-F[3]	+	-	+	+	+	+	+	+	+	+	+	+	+		
PKB(K-A)-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
PDK1-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
p70S6K-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
p70S6K(K-A)-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
Cdc42-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
Rac1-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
F[3]-FRAP	+	+	+	+	+	+	+	+	+	+	+	+	+		
F[3]-FRAP(D-A)	+	+	+	+	+	+	+	+	+	+	+	+	+		
PP2Ac-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
S6 protein-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
4EBP1-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		
FKBP-F[3]	+	+	+	+	+	+	+	+	+	+	+	+	+		

148 combinations tested • 65 potential interactions • 14 observed and 5 novel

Fig. 6B



DHFR PCA with Texas Red-MTX

Fig. 6C

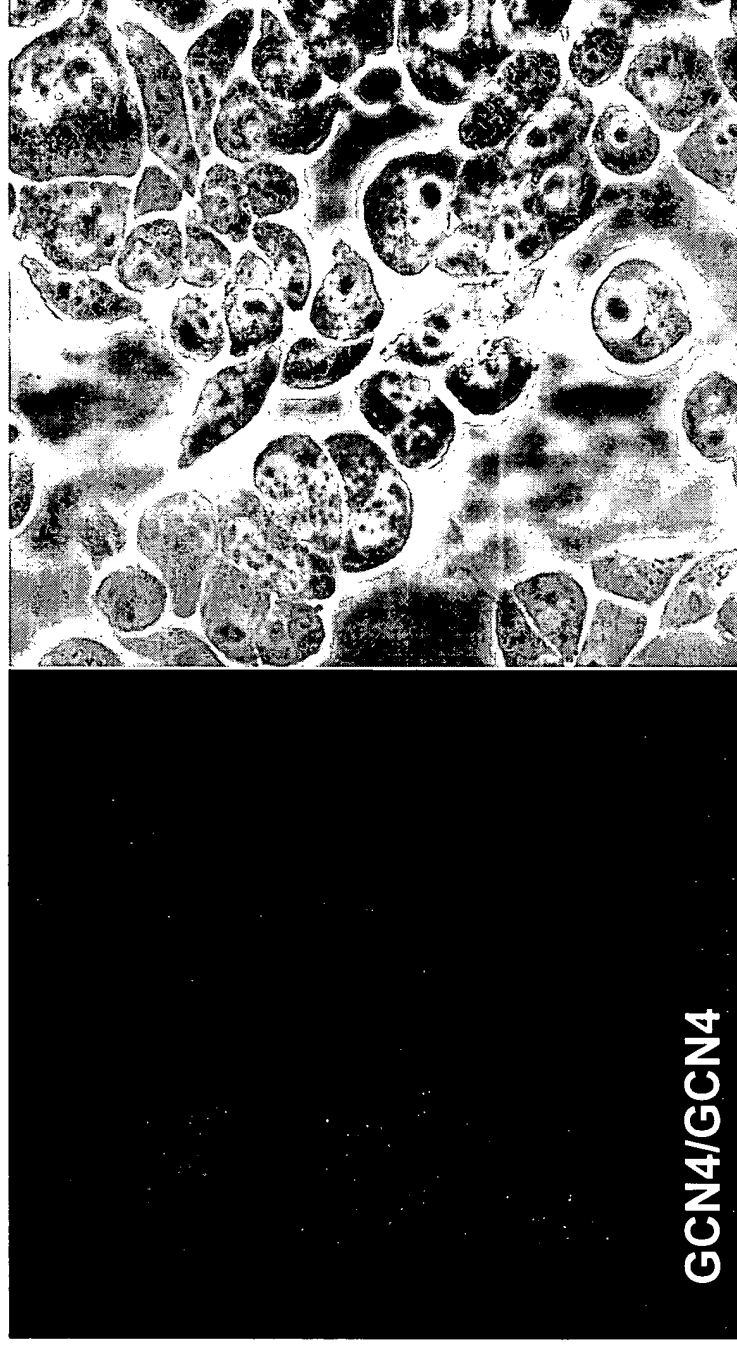


Fig. 7

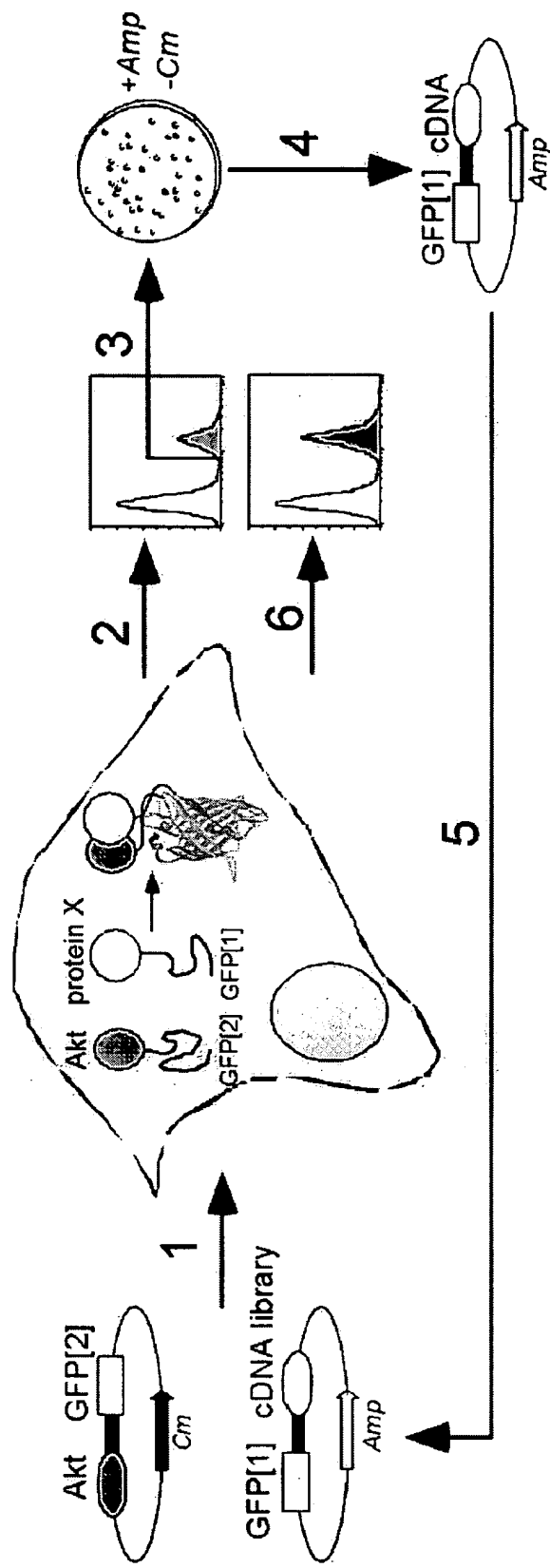
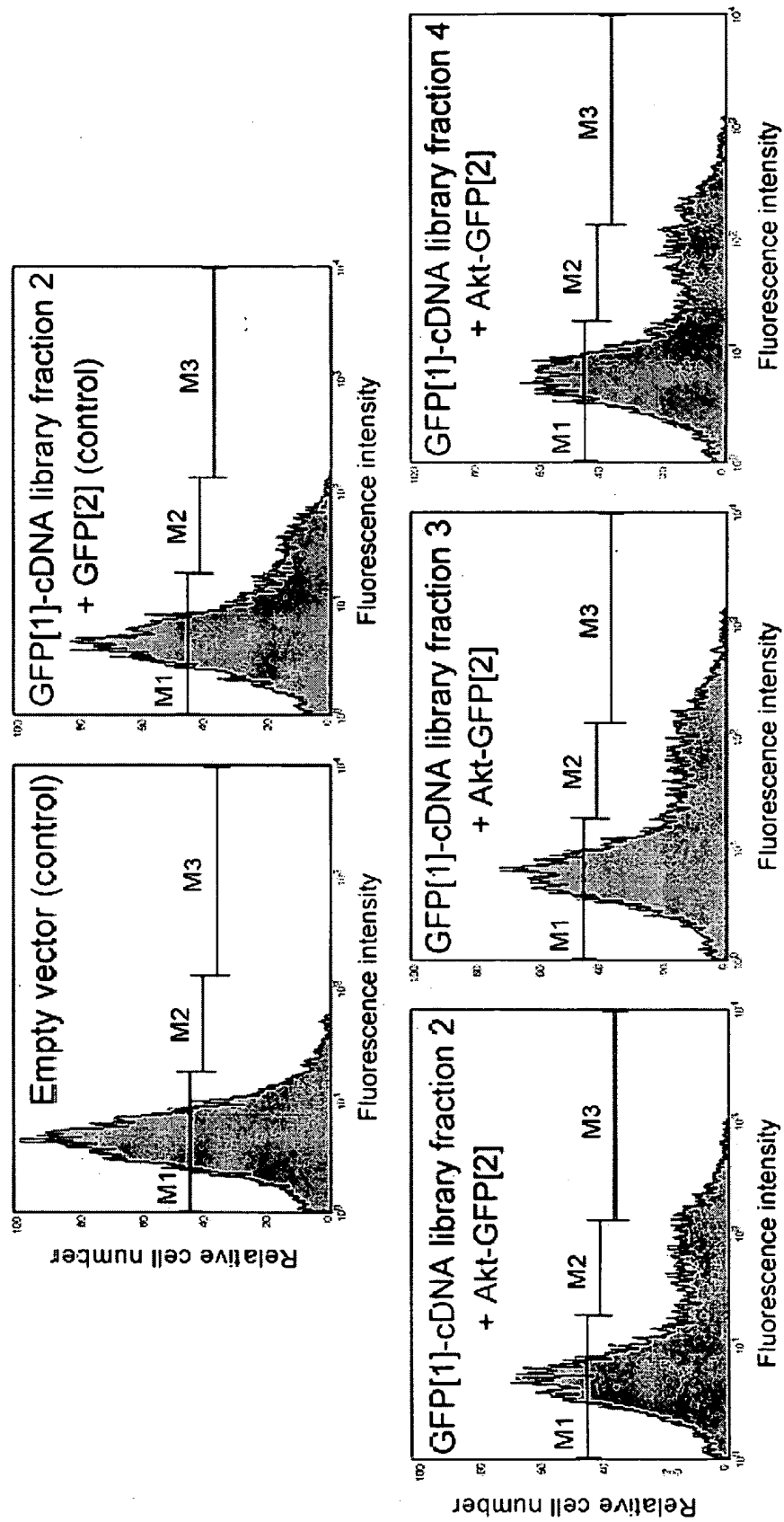


Fig. 8



A)

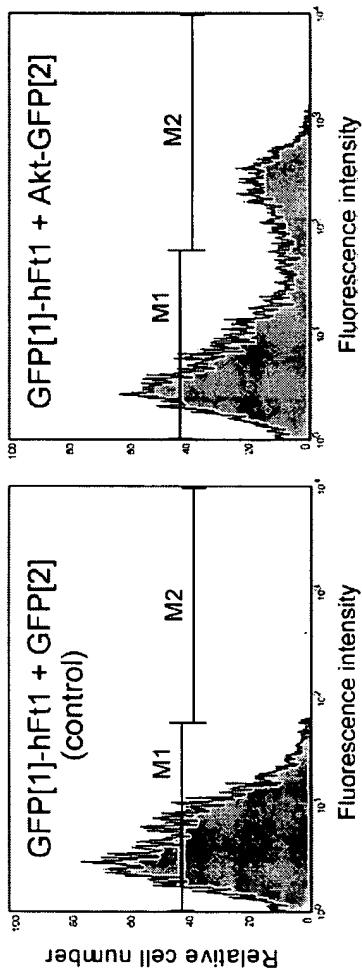
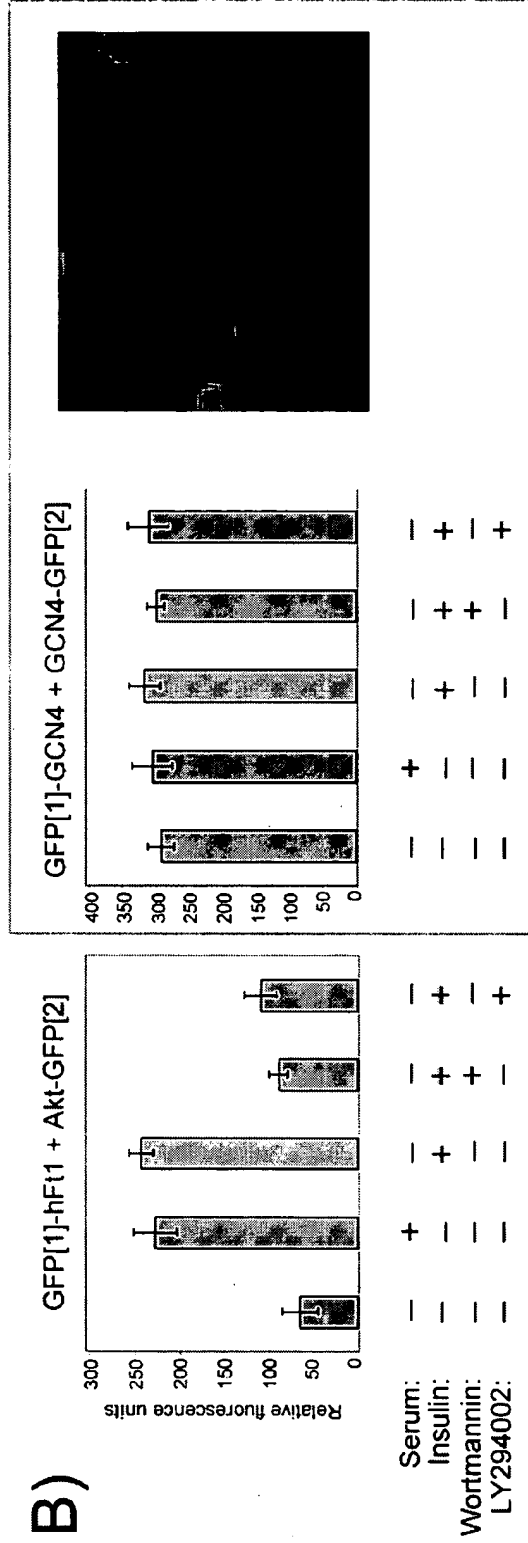


Fig. 9

B)



C)

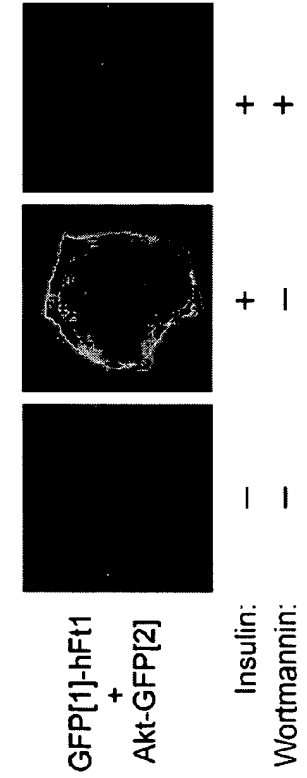


Fig. 10

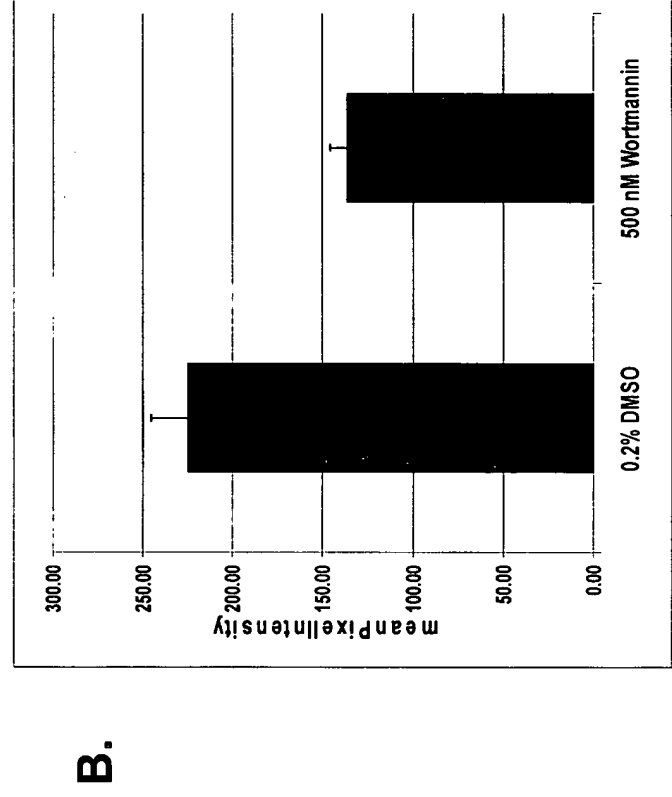
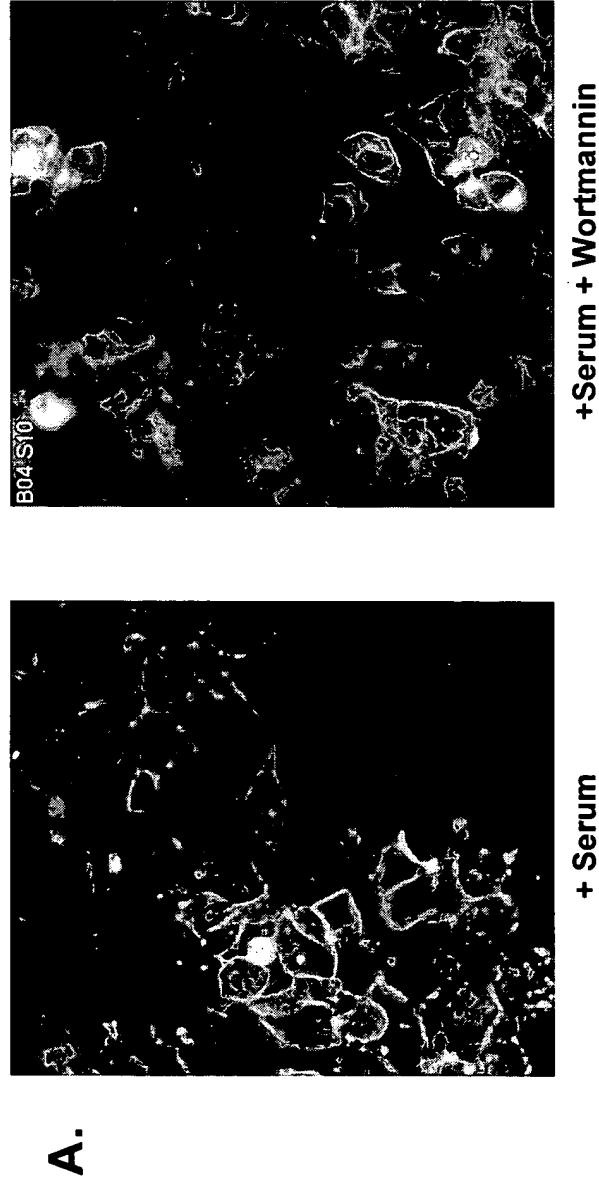
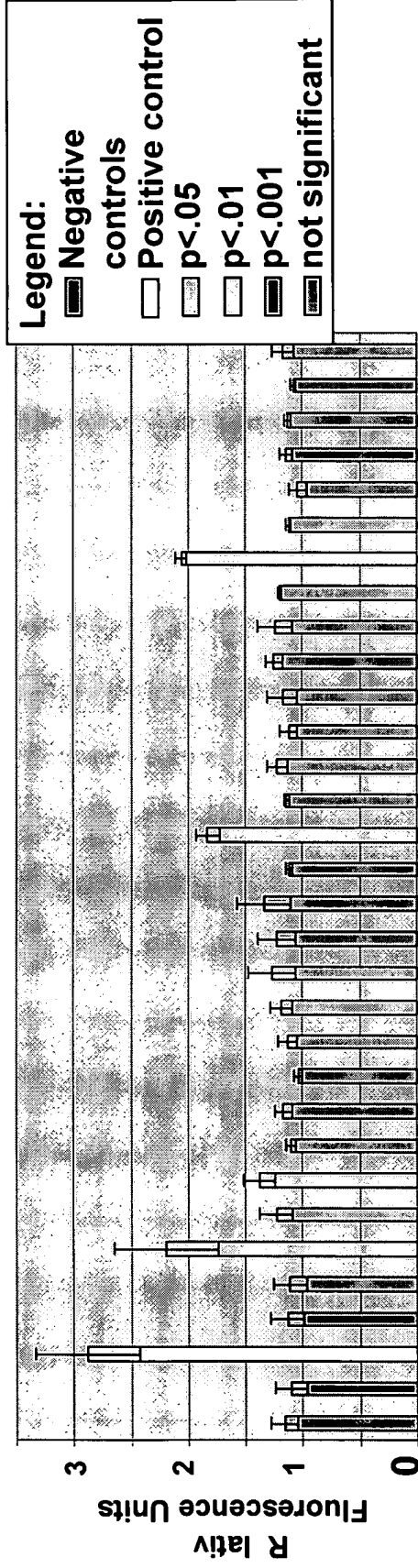


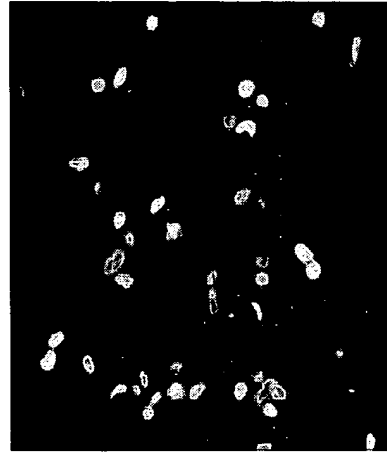
Fig. 11

(A)



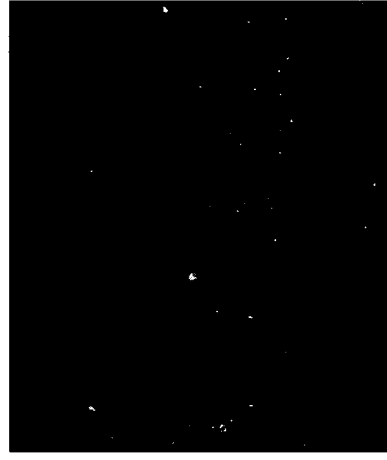
Microtiter well positions (triplicates)

(B)



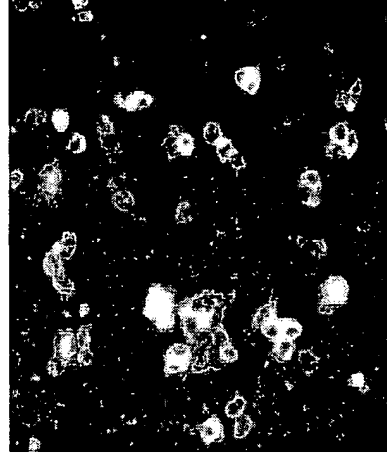
Positive control

(C)



Negative control

(D)



Novel interaction

Fig. 12

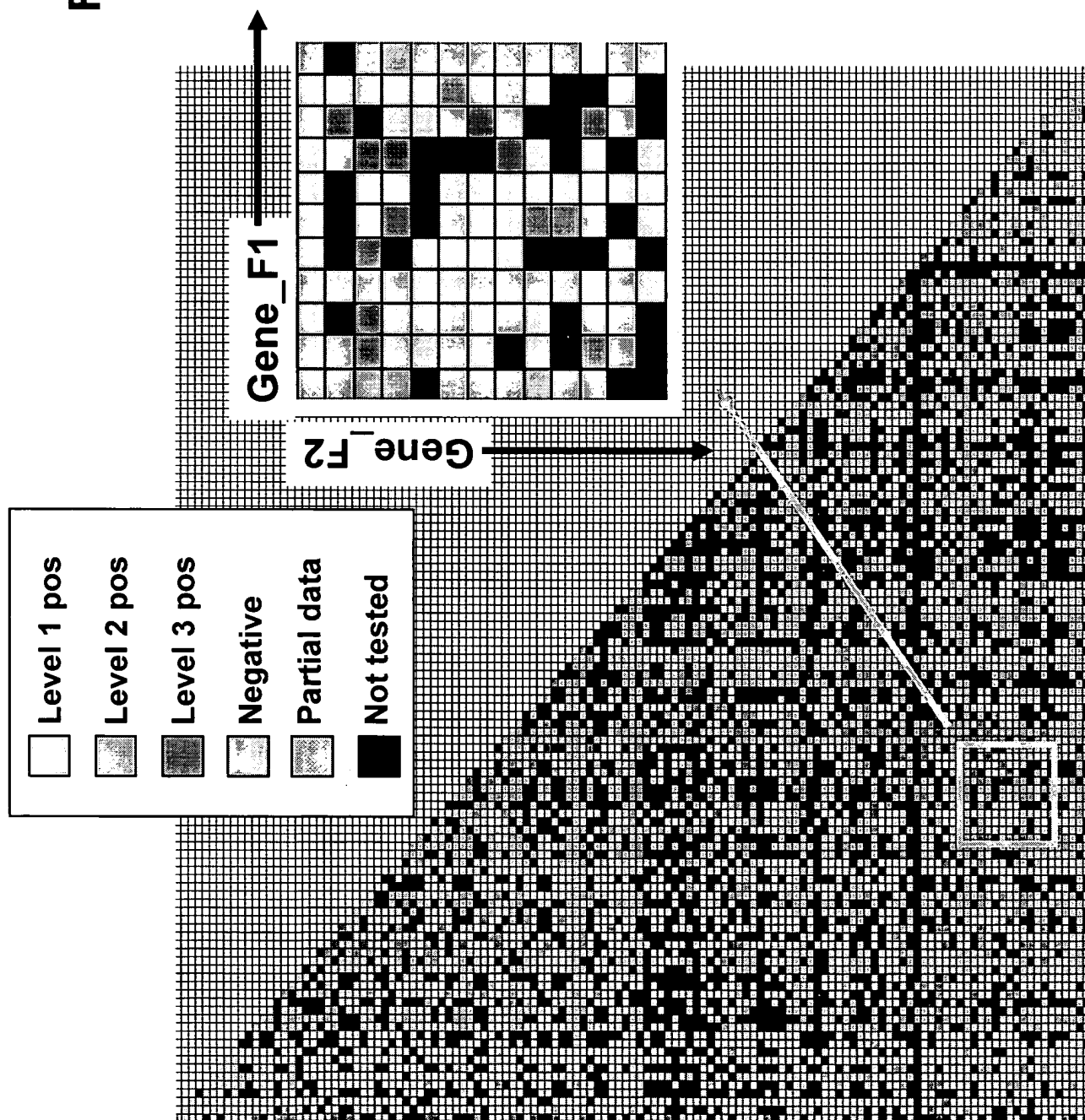


Fig. 13

	Working Definition	All Orientations	Unique
Assays in triplicate		9931 (29,793 single)	5773 (17,139 single)
Pos, Level 1	Plate reader $pval_{mean} \leq 0.001$ Image analysis score > 16 Fold increase ≥ 1.3	227 (2.3%)	205 (3.6%)
Pos, Level 2	Plate reader $pval_{mean} \leq 0.01$ Image analysis score > 8 Fold increase ≥ 1.2	1014 (10.2%)	730 (12.6%)
Pos, Level 3	Plate reader $pval_{mean} < 0.05$ Image analysis score ≥ 2 Fold increase > 1	994 (10.0%)	640 (11.1%)
Negative	Plate reader $pval_{mean} < 0$ or ≥ 0.05 Image analysis score < 2	6143 (61.9%)	3542 (61.4%)
Partial data	Plate reader or image data only	20 (0.2%)	19 (0.3%)
Discordant	Image and Plate reader scores discordant	1533 (15.4%)	637 (12.0%)

Fig. 14

	Positive Control	Negative Control	Single DNA	No DNA
Assays in triplicate	459	459	459	459
Pos, Level 1	159 (34.7%)	0 (0%)	0 (0%)	0 (0%)
Pos, Level 2	264 (57.5%)	0 (0%)	0 (0%)	0 (0%)
Pos, Level 3	13 (2.8%)	0 (0%)	1 (0.2%)	0 (0%)
Negative	6 (1.3%)	451 (98.3%)	423 (92.2%)	426 (92.8%)
Partial data	15 (3.3%)	8 (1.7%)	8 (1.7%)	8 (1.7%)
Discordant	2 (0.4%)	0 (0%)	27 (5.9%)	25 (5.5%)

Excluding Partial and Discordant Data:

True positives = 98.6%
 True negatives = 99.9%
 False positives = 0.08%
 False negatives = 1.4%

Fig. 15

